Modification of Casein by Phosphatases and Protein Kinases

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Research on the action of phosphatases and protein kinases on bovine caseins is reviewed, emphasizing the effect of these enzymes on the major fractions, α_{s1}-, β-, and κ-caseins. Two phosphatases (potato acid phosphatase and spleen phosphoprotein phosphatase), which differ in their catalytic behavior, can remove all the phosphate groups from α_{s1}-casein. Several properties of α_{s1}-casein are altered when the phosphate groups are removed. Dephosphorylated α_{s1}-casein has a higher isoionic point, has a decreased electrophoretic mobility at alkaline pH, and binds less calcium. Phosphate can be incorporated into casein by protein kinases which use ATP as the phosphate donor. Differences in the substrate specificity of two protein kinases (casein kinase from lactating mammary gland and cyclic AMP-dependent protein kinase from rabbit skeletal muscle) can be related to the primary structure of the individual caseins used as substrates. The data suggest that protein kinases are very specific in their substrate requirements, whereas phosphatases can act on all phosphoserine residues in casein.

Many of the unique properties of milk can be attributed to its major proteins, a group of acidic proteins known as casein. Although the caseins are dissimilar in many respects, they are predominantly phosphoproteins which occur in milk as colloidal particles containing up to 50000 monomers. The mechanism by which the monomers are assembled into colloids has been the subject of extensive research. The reader is referred to reviews by Waugh (1971) and Thompson and Farrell (1974). In all proposed models, ionic bonds between the phosphate groups of casein and calcium have been implicated in the assembly of casein components. Thus, the covalently bound phosphate of casein seems to be an important factor in maintaining the colloidal structure of milk proteins.

This paper provides selected examples from the literature of how phosphatases and protein kinases can modify casein by removing or adding phosphate groups. The availability of pure caseins makes it possible to relate the action of the enzymes to the primary structure of the molecule. We are beginning to learn the specific sites in casein which are phosphorylated by protein kinases. These studies should lead to an understanding of how to add phosphate groups to specific serine or threonine residues in casein and in nonmilk proteins.

CASEIN

Most bovine caseins have been purified to homogeneity and their sequences have been determined (Mercier et al., 1972). It is known that the major casein components consist of α_{s1}-, β-, and κ-casein, all of which exist in polymorphic forms (Thompson and Farrell, 1974). α_{s1}-Casein contains eight phosphate groups, β-casein five phosphate groups, and κ-casein one phosphate group. Several excellent reviews summarize recent developments in research on casein (Swaisgood, 1973; Rose et al., 1970; McKenzie, 1967, 1971) and phosphoproteins (Taborsky, 1974). Because α_{s1}-casein is the major protein in cow's milk and has been well characterized, it has been used as a model for studies on phosphatases and protein kinases.

The complete primary structure of α_{s1}-casein (Figure 1) has been established (Mercier et al., 1971; Grosclaude et al., 1973) and shown to be a single polypeptide chain containing 199 amino acid residues with a molecular weight of 23616. The high proline content (8.5%) and its even distribution suggest that α_{s1}-casein has no secondary structure, as proline residues prevent helix formation in proteins. α_{s1}-Casein contains no cysteine; thus disulfide bonds cannot occur.

α_{s1}-Casein has eight phosphate groups attached to specific serine residues and eight additional serines and three threonines that are not phosphorylated. Four out of the eight phosphoserines are located in an acidic section of the polypeptide chain, residues 62 through 71. A similar peptide is found in β-casein (Mercier et al., 1971) and human casein (Greenberg et al., 1976), suggesting that these regions must be relevant to the properties of caseins.

DEPHOSPHORYLATION OF α_{s1}-CASEIN BY PHOSPHATASES

A variety of phosphatases have been used to remove the phosphate groups from casein. Enzymes that have been used for this purpose include citrus fruit enzymes (Mecham and Olcott, 1949), milk alkaline phosphatase (Zittle and Bingham, 1959), spleen phosphoprotein phosphatase (Sundararajan and Sarma, 1957; Bingham et al., 1972b), and potato acid phosphatase (Hsu et al., 1958; Bingham et al., 1976). Although steric factors and amino acid sequences near the phosphate groups may affect the rate of dephosphorylation, the various phosphatases seem to work effectively, an indication that all the phosphate groups in casein are accessible to phosphatases. There is little evidence that certain phosphate groups are cleaved more readily than others. In fact, the removal of phosphate groups from β-casein by almond phosphatase has been shown to occur randomly (Lerch et al., 1975). The accessibility of the phosphate groups in casein to phosphatase hydrolysis seems to be due to the unstructured conformation of the casein molecule.

Totally dephosphorylated α_{s1}-casein has been prepared using spleen phosphoprotein phosphatase and potato acid phosphatase, two enzymes which differ markedly in their catalytic properties (Table I). Both enzymes act on phosphoproteins (α_{s1}-casein and β-casein), the phosphopeptide from β-casein (molecular weight of 3000), and *p*-nitrophenyl phosphate. The two enzymes differ in their activity toward ATP. ATP, as well as inorganic pyrophosphatases, are excellent substrates for spleen phosphoprotein phosphatase, but are not hydrolyzed by potato acid phosphatase. Differences between the two enzymes are also evident when phosphoserine is used as a substrate. Although the phosphate groups in casein occur as phosphoserine, spleen phosphoprotein phosphatase has little activity toward this substrate and other phosphomonoesters (Hofman, 1958b; Revel and Racker, 1960). On the other hand, potato acid phosphatase hydrolyzes

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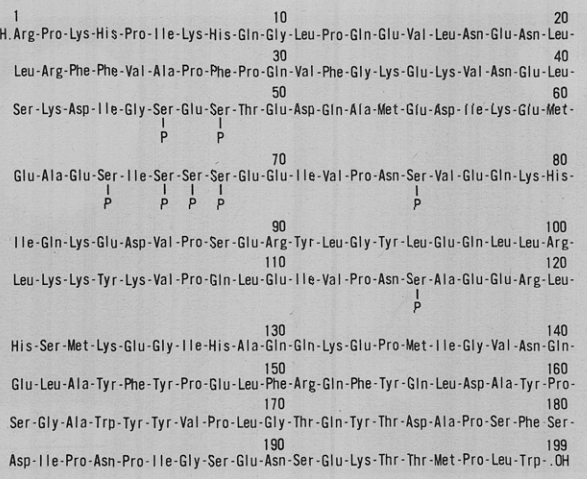

Figure 1. The primary structure of bovine α_{s1}-casein B according to Mercier et al. (1971) and Grosclaude et al. (1973).

Table I. Properties of Spleen Phosphoprotein Phosphatase and Potato Acid Phosphatase

Properties	Spleen phosphoprotein phosphatase[a]	Potato acid phosphatase[b]
Substrates hydrolyzed	Rel Act.	
α_{s1}-Casein	100	100
β-Casein	90	115
Phosphopeptide	52	54
p-Nitrophenyl phosphate	>400	550
ATP	600	0
Phosphoserine	0.5	72
Optimum pH	5.0–5.5	6.0–7.0
Activators	Reducing agents	None

[a] Data were summarized from material taken from Hofman (1958a,b) and Revel and Racker (1960). [b] Data taken from Bingham et al. (1976) with the exception of the ATP data, which were taken from Lora-Tamayo et al. (1962).

phosphoserine as well as glycerol phosphate and sugar phosphates (Lora-Tamayo et al., 1962), indicating that it is a typical phosphomonoesterase. There is no satisfactory explanation for the unique catalytic specificity of spleen phosphoprotein phosphatase, which can remove the phosphate groups from phosphoserines in proteins, but not from free phosphoserine.

The optimum pH for spleen phosphoprotein phosphatase occurs from pH 5.0 to 5.5 (Table I). Since α_{s1}-casein is not soluble in this pH range, most investigators remove the phosphate groups from casein at pH 6.0 where casein is soluble. However, dephosphorylated casein is insoluble at pH 6.0 and precipitates from the reaction mixture. Although Sundararajan and Sarma (1957) consider the precipitation to be an advantage in the isolation of dephosphorylated casein, the reaction is more difficult to control. Potato acid phosphatase has optimal activity toward α_{s1}-casein from pH 6.0 to pH 7.0. By using potato acid phosphatase at pH 7.0, problems due to the insolubility of casein are avoided.

Spleen phosphoprotein phosphatase requires reducing agents, such as β-mercaptoethanol or ascorbic acid, for maximum activity whereas potato acid phosphatase does not require activators.

Although both enzymes can be used to prepare dephosphorylated α_{s1}-casein, potato acid phosphatase has several advantages over spleen phosphoprotein phosphatase in that it is active at pH 7.0, requires no known activators, and can be obtained commercially in a highly purified form.

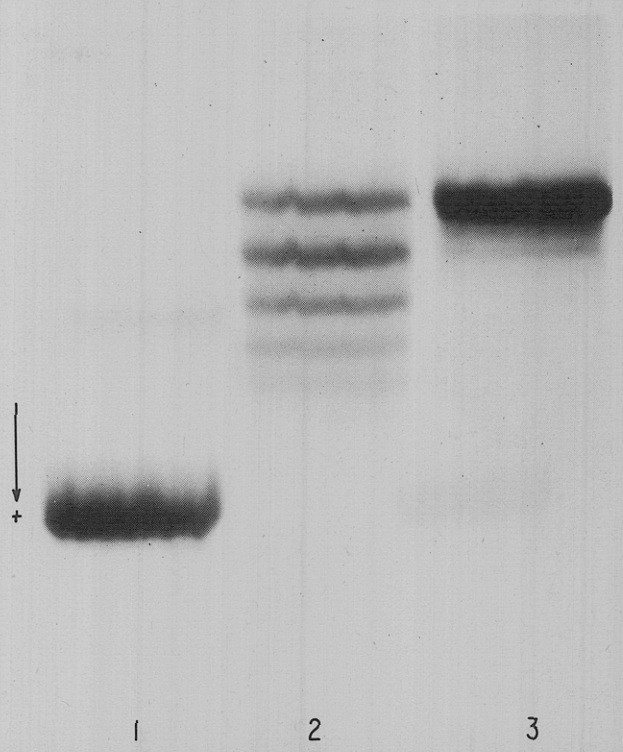

Figure 2. Polyacrylamide gel electrophoresis (pH 9.2) of α_{s1}-casein as a function of time of incubation with spleen phosphoprotein phosphatase. Incubation times were 0 (1), 1.5 (2), and 5 (3) h (reproduced from Bingham et al., 1972b).

Electrophoretic techniques have been valuable in detecting the extent of dephosphorylation (Bingham et al., 1972b, 1976; Yoshikawa et al., 1974). Alkaline polyacrylamide gel patterns, illustrating the effect of spleen phosphoprotein phosphatase on α_{s1}-casein, are shown in Figure 2. As the dephosphorylation proceeds, new bands with slower mobilities are observed. This heterogeneity represents α_{s1}-casein with varying amounts of phosphate. At longer incubation times, the multiple bands disappear and the dephosphorylated α_{s1}-casein is a single bond. A study of various parameters indicates that spleen phosphoprotein phosphatase is most effective at a low α_{s1}-casein concentration, 1–3 mg/ml. At higher substrate concentrations, the reaction does not go to completion. Considerable phosphate inhibition is evident when potato acid phosphatase is used to dephosphorylate α_{s1}-casein. Therefore, a dialysis between incubations was needed to produce homogeneous dephosphorylated α_{s1}-casein (Figure 3).

These studies indicate that complete dephosphorylation of casein can be obtained by careful attention to parameters affecting the reaction. Thus, the failure of certain phosphatases to remove all the phosphate from phosphoproteins may result from either the inherent specificity of the phosphatase or the unfavorable reaction conditions employed.

CHARACTERISTICS OF DEPHOSPHORYLATED CASEIN

Native α_{s1}-casein is an acidic protein with an estimated net charge of −24 at pH 6.6 (Waugh et al., 1971; Swaisgood, 1973). If the eight phosphate groups are removed, the dephosphorylated α_{s1}-casein has a charge of −9.5. The slower mobility of dephosphorylated α_{s1}-casein on poly-

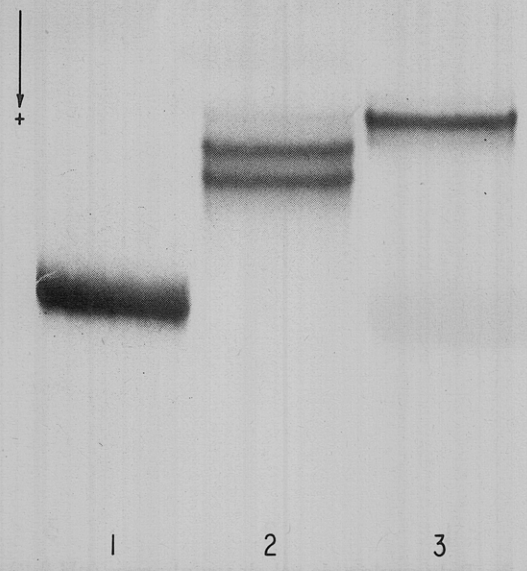

Figure 3. Polyacrylamide gel electrophoresis (pH 9.2) of α_{s1}-casein (1); α_{s1}-casein incubated with potato acid phosphatase for 2 h (2); α_{s1}-casein incubated with potato acid phosphatase for 2 h followed by dialysis against water for 65 h (3) (Bingham et al., 1976); reproduced with the permission of *Biochimica et Biophysica Acta*.

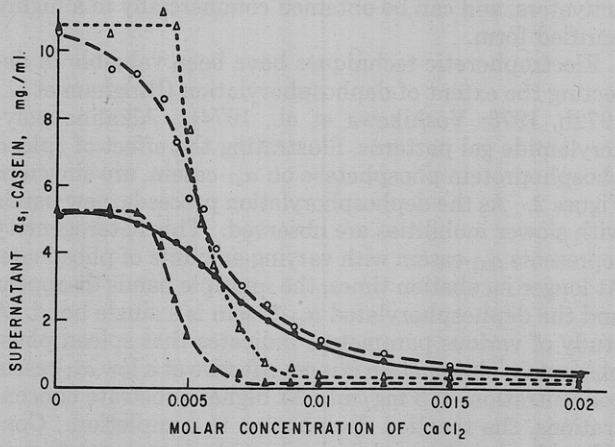

Figure 4. The solubility of α_{s1}-casein as a function of increasing $CaCl_2$ concentration: (△) α_{s1}-casein (10.6 mg/ml); (○) dephosphorylated α_{s1}-casein (10.6 mg/ml); (▲) α_{s1}-casein (5.3 mg/ml); (●) dephosphorylated α_{s1}-casein (5.3 mg/ml). The solutions contained 0.07 M KCl and 0.01 M imidazole-HCl buffer (pH 7.0) (reproduced from Bingham et al., 1972b).

acrylamide gels reflects the decrease in negative charge (Figures 2 and 3). In addition, the removal of phosphate groups from α_{s1}-casein increases the isoionic point from 4.9 to 5.9 (Bingham et al., 1972b).

One of the characteristic features of α_{s1}-casein is its insolubility in the presence of calcium ions (0.01 M). Cross-linking of adjacent polymers through calcium has been proposed as a likely mechanism for this precipitation (Waugh et al., 1971). When the phosphate groups are removed from α_{s1}-casein, the protein retains its ability to precipitate in the presence of calcium ions (Figure 4). Yamauchi et al. (1967) reported similar results using

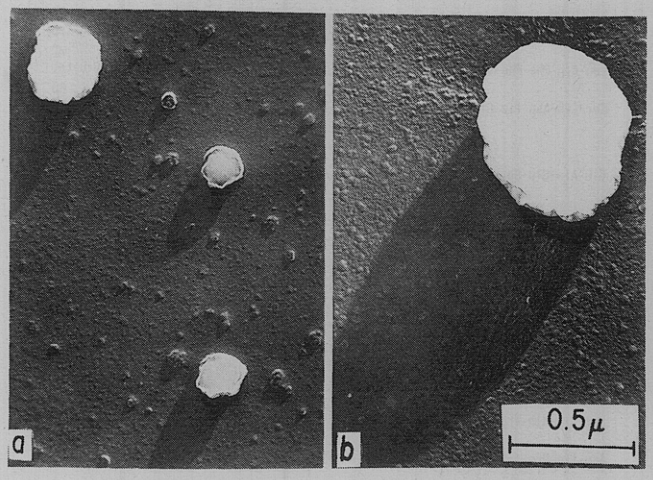

Figure 5. Electron micrograph of simulated milk micelles. Solutions contained 1% α_{s1}-casein, 0.125% κ-casein, 0.01 M $CaCl_2$, and 0.01 M imidazole buffer (pH 7.0): (a) α_{s1}-casein; (b) dephosphorylated α_{s1}-casein. Micrographs are reproduced from Bingham et al. (1972b).

dephosphorylated whole casein as well as dephosphorylated α_{s1}-casein. Therefore, the binding of calcium to the phosphate groups of α_{s1}-casein does not seem essential for precipitation. Unlike α_{s1}-casein, the calcium-dependent precipitation of β-casein is completely lost when the phosphate groups are removed and dephosphorylated β-casein is soluble in the presence of calcium ions (Yoshikawa et al., 1974). There is no satisfactory explanation for the difference between the two proteins.

Several studies support the concept that dephosphorylated α_{s1}-casein can bind calcium. According to Dickson and Perkins (1971), who used the radioisotope ^{47}Ca, dephosphorylated α_{s1}-casein binds 1.2 mol of calcium compared to native α_{s1}-casein which binds 8.5 mol. Yamauchi et al. (1967) estimated that dephosphorylated α_{s1}-casein retains 30% of the calcium binding sites found in native α_{s1}-casein. Ho and Waugh (1965), using infrared spectroscopy, pointed out that the phosphate groups in casein are the primary but not the sole calcium binding sites. Therefore, the organic phosphate groups of α_{s1}-casein account for some but not all of the calcium binding sites.

Although α_{s1}-casein is insoluble in the presence of calcium ions, κ-casein can prevent precipitation through the formation of colloidal micelles (Waugh, 1971). Pepper and Thompson (1963) showed that dephosphorylation of α_{s1}-casein impaired its ability to form micelles with κ-casein in 0.02 M $CaCl_2$ at pH 7.0. At a lower $CaCl_2$ concentration (0.01 M) and a higher κ-casein concentration, Bingham et al. (1972b) succeeded in making micelles from dephosphorylated α_{s1}-casein. However, these micelles differed from normal micelles. Viewed in the electron microscope, the micelles were three times the size of native α_{s1}-casein micelles and fewer in number (Figure 5). The addition of 0.01 M KCl completely inhibited the formation of dephosphorylated α_{s1}-casein micelles, but had no effect on micelles made with native α_{s1}-casein. These investigations also suggest that calcium binding sites other than phosphate are involved in the formation of dephosphorylated α_{s1}-casein micelles and that the forces involved in binding are weaker.

PHOSPHORYLATION BY PROTEIN KINASES

Since phosphate groups affect the properties of casein, it is important to understand how the phosphate groups are incorporated into casein. The mechanism by which proteins are phosphorylated was first described by Burnett

Table II. Phosphorylation of Proteins by Casein Kinase[a]

Protein substrate	Rate of phosphate addition, nmol/mg per 20 min
α_{s1}-Casein	8.6
Dephosphorylated α_{s1}-casein	43.0
β-Casein	2.5
Dephosphorylated β-casein	32.4
κ-Casein	1.3
Dephosphorylated κ-casein	8.5
β-Lactoglobulin	2.1
α-Lactalbumin	1.1
Fat globule membrane proteins	6.1
Histone-arginine rich	0.1
Histone-lysine rich	0.1
Phosvitin	0.3
Lysozyme	0

[a] Data taken from Bingham and Farrell (1974); reproduced with the permission of the *Journal of Biological Chemistry*.

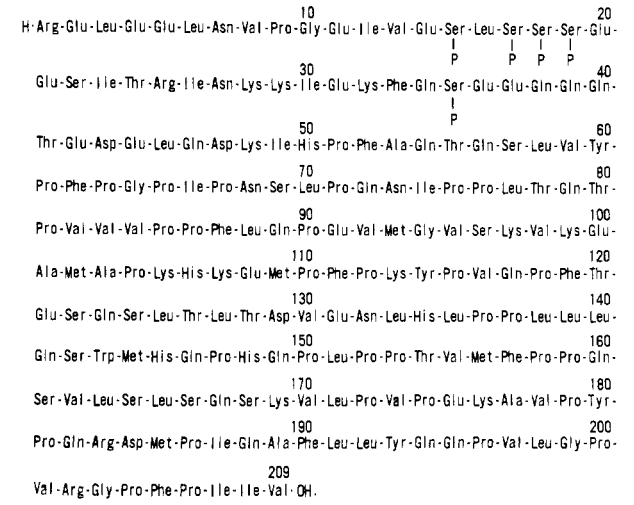

Figure 6. The primary structure of β-casein A^2 (Ribadeau Dumas et al., 1972).

and Kennedy (1954), who used rat liver mitochondria as an enzyme source. These investigators showed that the phosphorylation of proteins is catalyzed by a protein kinase, which uses the terminal phosphate of ATP as the

$$\text{protein} + \text{ATP} \xrightarrow{Mg^{2+}} \text{protein-P} + \text{ADP}$$

phosphate donor in the presence of divalent cations (usually Mg^{2+}). Of the protein substrates tested, only casein was phosphorylated by this enzyme. α-Casein (a mixture of α_{s1}-casein and κ-casein) was phosphorylated at four times the rate of β-casein. Phosphate incorporation was drastically reduced when the α-casein was dephosphorylated with alkali.

Research on protein kinases has expanded rapidly following the observation by Walsh et al. (1968) that certain protein kinases are stimulated by adenosine 3′,5′-monophosphate (cyclic AMP). Because of the emphasis on cyclic AMP in protein kinase research, protein kinases are classified as cyclic AMP dependent and cyclic AMP independent (Traugh et al., 1974). Two excellent reviews summarize recent developments in protein kinase research (Walsh and Krebs, 1973; Rubin and Rosen, 1975).

This article will describe the phosphorylation of casein by two distinctly different protein kinases, casein kinase from lactating rat mammary gland and cyclic AMP-dependent protein kinase from rabbit skeletal muscle.

CASEIN KINASE

The biosynthesis of casein occurs in the lactating mammary gland. Turkington and Topper (1966) provided evidence that the biosynthesis occurs in two steps—synthesis of the polypeptide chain, followed by phosphate addition. Support for this mechanism was provided by Bingham et al. (1972a), who isolated an enzyme, casein kinase, which catalyzes the second step, namely, the addition of phosphate to dephosphorylated casein. Casein kinase is found in the Golgi apparatus region of lactating mammary gland cells. The casein kinase from rat mammary gland has been characterized (Bingham and Farrell, 1974). Like other protein kinases, the enzyme catalyzes the phosphate incorporation into proteins using ATP as a phosphate donor in the presence of certain divalent cations. An unusual feature of casein kinase is that Ca^{2+} can replace Mg^{2+} as the divalent cation; most protein kinases are inhibited by Ca^{2+} (Kuo et al., 1970). Cyclic AMP has no effect on activity, which classifies casein kinase as a cyclic AMP-independent protein kinase (Traugh et al., 1974). Substrate specificity experiments indicate a marked preference of casein kinase for dephosphorylated caseins over native caseins (Table II).

Chew and Mackinlay (1974) have prepared a casein kinase from lactating bovine mammary gland which appears to phosphorylate the serine residues in α_{s1}-casein that are normally phosphorylated in vivo. Their casein kinase does not phosphorylate β-casein and, therefore, differs from the casein kinase of Bingham and Farrell (1974).

The fact that casein kinases preferentially phosphorylate dephosphorylated caseins suggests that the site of phosphorylation is on a serine from which the phosphate has been removed. Chew and Mackinlay (1974) indicated that their enzyme phosphorylates serines, 64 to 68, in the α_{s1}-casein molecule (see Figure 1), but were unable to show incorporation of phosphate in the four other serine residues of α_{s1}-casein. At the present time, quantitative data on the amount of phosphate incorporated by casein kinase and the exact location of the phosphorylated serines are lacking, although research in this area should be fruitful in the future.

A plausible theory for the mechanism of casein kinase action has been proposed by Mercier et al. (1972). Their theory is based on comparisons of the sequence of amino acids surrounding the phosphorylated and phosphate-free hydroxy amino acid residues in the three main caseins. α_{s1}-Casein B (Figure 1) has 16 serine residues, 8 of which are phosphorylated. Every phosphorylated serine in this molecule has a glutamic acid or a phosphoserine two residues to the right in the sequence. This configuration does not occur when the serines are phosphate free. The α_{s1}-casein D variant with an additional phosphate group has a phosphothreonine, which replaces alanine-53. There is a glutamic acid residue two residues to the right of this phosphorylated site. β-Casein A^2 has 16 serines, 5 of which are phosphorylated, and 9 threonines. Examination of Figure 6 shows that all serines and threonines in β-casein A^2 conform to Mercier's theory. β-Casein C, which has only 4 phosphate groups, differs from β-casein A^2 in that glutamic acid at position 37 is replaced by lysine and serine-35 is not phosphorylated. The lack of phosphate on serine-35 provides additional support for the concept that phosphorylation depends on the presence of either phosphoserine or glutamic acid two residues to the right in the sequence. Thus, casein kinase recognizes a phosphorylation site corresponding to the tripeptide sequence Ser/Thr-X-Glu/SerP, where X is any amino acid. The

Table III. Selected Sequences in Casein Polymorphs[a]

	Location
Sequences containing phosphate group	
46	
SerP-Glu-SerP-Thr-Glu-Asp	α_{s1}-Casein A, B, C, D
53	
ThrP-Met-Glu-Asp	α_{s1}-Casein D
64	
SerP-Ile-SerP-SerP-SerP-Glu-Glu-Ile	α_{s1}-Casein A, B, C, D
75	
SerP-Val-Glu-Gln	α_{s1}-Casein A, B, C, D
115	
SerP-Ala-Glu-Glu	α_{s1}-Casein A, B, C, D
15	
SerP-Leu-SerP-SerP-SerP-Glu-Glu-Ser	β-Casein A^1, A^2, A^3, B, C
35	
SerP-Glu-Glu-Gln	β-Casein A^1, A^2, A^3, B
35	
SerP-Lys-Glu-Gln	β-Casein E
149	
SerP-Pro-Glu-Val	κ-Casein A, B
Potential sites for phosphorylation[b]	
127	
Ser-Gly-Glu-Pro	κ-Casein A, B
135	
Thr-Ile-Glu-Ala	κ-Casein B
135	
Thr-Thr-Glu-Ala	κ-Casein A
145	
Thr-Leu-Glu-Ala	κ-Casein B
145	
Thr-Leu-Glu-Asp	κ-Casein A

[a] Sequence data were obtained from the following sources: α_{s1}-casein A, B, C, and D (Mercier et al., 1971; Grosclaude et al., 1973); β-casein A^1, A^2, A^3, B, and C (Grosclaude et al., 1972); β-casein E (Grosclaude et al., 1974); and κ-casein A and B (Mercier et al., 1973). [b] These are peptides containing Ser/Thr-X-Glu/SerP sequences, in which the serine is not phosphorylated.

genetic forms of α_{s1}-casein (A, B, C, and D) and β-casein (A^1, A^2, A^3, B, C, and E) all support this hypothesis (Table III).

However, other milk proteins do not conform to the proposed theory. β-Lactoglobulin has three potential sites for phosphorylation, yet the molecule contains no phosphate (Braunitzer et al., 1972). κ-Casein has four potential sites, Ser-127, Thr-135, Thr-145, and Ser-149; only Ser-149 is phosphorylated (Table III). Mercier et al. (1972) postulate that a steric hindrance to phosphorylation may be due to the tertiary configuration of κ-casein or to the presence of carbohydrate moieties on the hydroxy amino acid residues.

Since glutamic acid and phosphoserine are acidic residues, acidity near the phosphorylated residue may also be an important factor as suggested by Mano and Imahori (1970). Examination of Table III shows that a second acidic residue (glutamic acid, aspartic acid, or phosphoserine) occurs in most of the peptides adjacent to the phosphoserine or glutamic acid in the N + 2 position. Sequence data give some support to the idea that double acids may be the specificity determinant. It may be significant that double acids occur near all the phosphorylated sites in α_{s1}-casein A, B, C, and D, and β-casein A^1, A^2, A^3, B, C, and E, with two exceptions, serine-75 in α_{s1}-casein and serine-35 in β-casein E. The double acid theory would explain the lack of phosphate groups in β-lactoglobulin and κ-casein. It does not explain why serine-149 in κ-casein A and B is phosphorylated or why threonine-145 in κ-casein A, which contains a double acid (Glu-Asp), is not phosphorylated. Although examination of sequence data has merit, the specificity of casein kinase will ultimately be determined by examining its action on substrates consisting of proteins and peptides with known sequences.

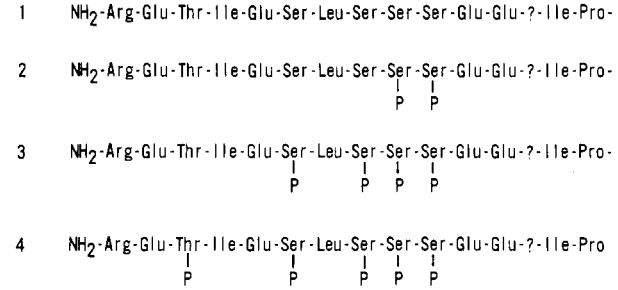

Figure 7. The primary structure of the N-terminal end of human casein containing 0 (1), 2 (2), 4 (3), and 5 (4) phosphate groups per monomer according to Greenberg et al. (1976).

If caseins are derived from unphosphorylated polypeptides, there must be an order in their phosphorylation. The Ser-X-Glu must be phosphorylated to SerP-X-Glu in order to generate phosphoserines, which can then act as specificity determinants. An interesting model for this idea is human casein, which has been found to exist in multiphosphorylated forms, containing 0 to 5 phosphate groups per molecule (Groves and Gordon, 1970). All the phosphate groups are located in the first 15 residues of the molecule (Greenberg et al., 1976). Figure 7 shows the sequence and location of the phosphate groups in human casein, containing 0, 2, 4, and 5 phosphates per mole. The fact that phosphates are located on specific residues, rather than randomly, suggests that human casein is synthesized by stepwise phosphorylation. However, dephosphorylation of the fully phosphorylated form must be considered as a possibility. Whether this has any relevance for bovine casein is not known, as bovine casein is always found fully phosphorylated.

CYCLIC AMP-DEPENDENT PROTEIN KINASE

Unlike casein kinase, which seems to be very specific in its substrate requirements, cyclic AMP-dependent protein kinases phosphorylate a wide variety of substrates, including whole casein. Kemp et al. (1975) have recently studied the substrate specificity of the cyclic AMP-de-

Table IV. Phosphorylation of β-Casein Genetic Variants by Cyclic AMP-Dependent Protein Kinase[a]

Substrate β-casein variant	Rate of phosphate addition, nmol/min per mg	Amino acid residues in the variable positions[b]			
		37	67	106	122
A^1	17.1	Glu	His	His	Ser
A^2	21.5	Glu	Pro	His	Ser
A^3	14.6	Glu	Pro	Gln	Ser
B	1425.6	Glu	His	His	Arg
C	37.5	Lys	His	His	Ser

[a] Data taken from Kemp et al. (1975). [b] Assignments according to Grosclaude et al. (1972).

pendent protein kinase from rabbit skeletal muscle. They examined homogeneous casein samples and have determined the phosphorylated sites. When genetic variants of β-casein were tested as substrates, one variant, β-casein B, was phosphorylated approximately 70-fold more rapidly than the most common variant, β-casein A^2 (Table IV). The various β-caseins differ by known amino acid substitutions. The unique feature of β-casein B is that serine-122 is replaced by an arginine. The arginine replacement makes it possible for serine-124 to be phosphorylated. Kemp et al. (1975) suggested that arginine, 2 to 5 residues on the N-terminal side of serine, is the specific determinant common to the phosphorylation sites recognized by cyclic AMP-dependent protein kinases. Native and dephosphorylated α_{s1}-casein B were not phosphorylated by the skeletal muscle cyclic AMP-dependent protein kinase, but κ-casein was phosphorylated (Bylund and Krebs, 1974). There are two serines in κ-casein which could serve as potential sites for cyclic AMP-dependent protein kinase. As β-casein B is relatively rare in milk, it seems probable that κ-casein is the protein phosphorylated when whole casein is used as a substrate.

CONCLUDING REMARKS

Two phosphatases (spleen phosphoprotein phosphatase and potato acid phosphatase), which differ markedly in their catalytic properties, can remove all the phosphate groups from α_{s1}-casein. Protein kinases can add phosphate groups to specific serines (or threonines) in casein. The phosphorylation of casein seems to depend on its primary structure. The specificity determinant for casein kinase appears to be glutamic acid or phosphoserine; thus, this enzyme can phosphorylate dephosphorylated caseins. Cyclic AMP-dependent protein kinase can phosphorylate κ-casein and β-casein B, but not α_{s1}-casein (native or dephosphorylated) or β-caseins A^1, A^2, A^3, and C. Specificity studies suggest that cyclic AMP-dependent protein kinase recognizes arginine, 2 to 5 residues from the phosphorylated serine.

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